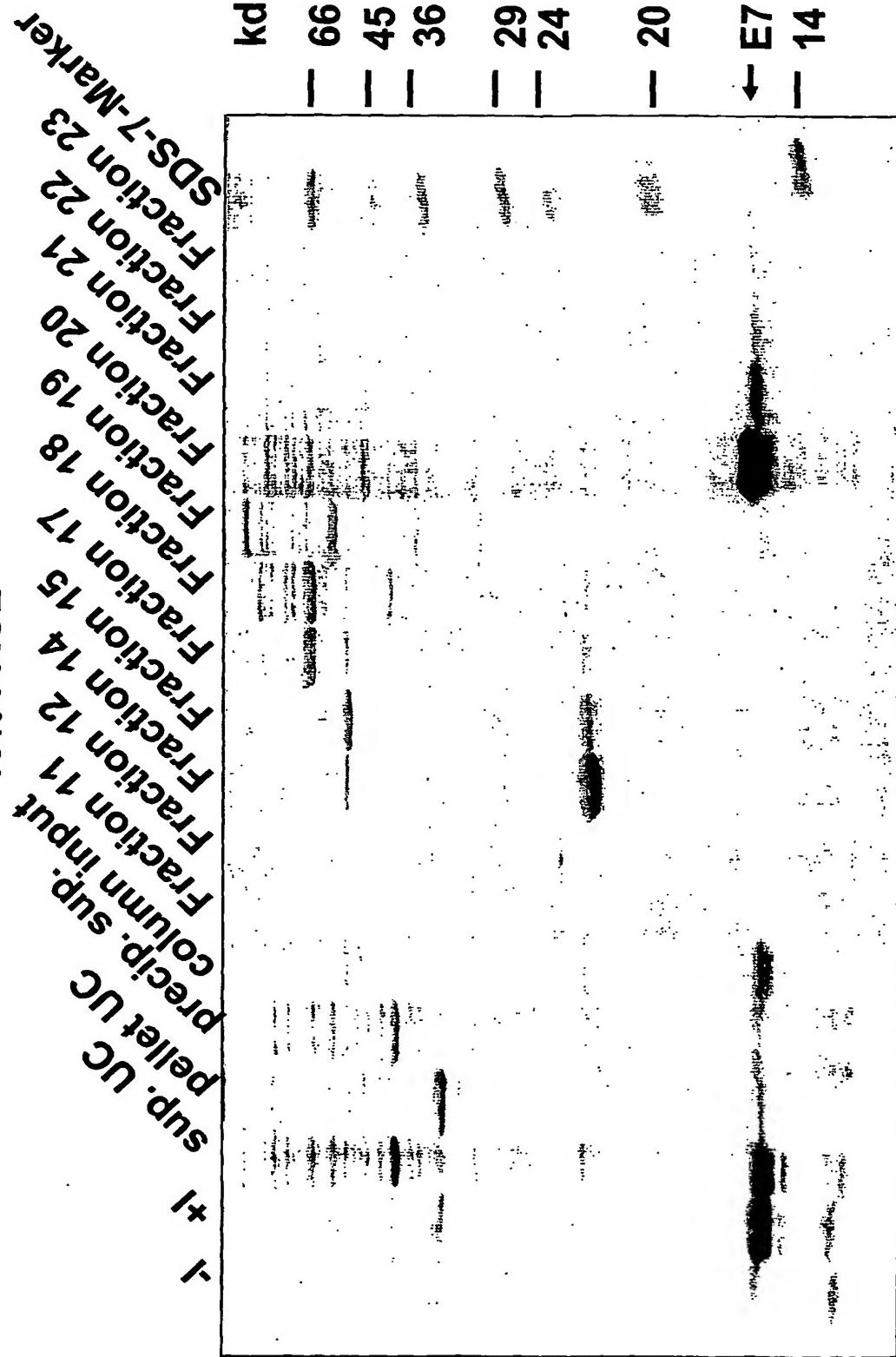
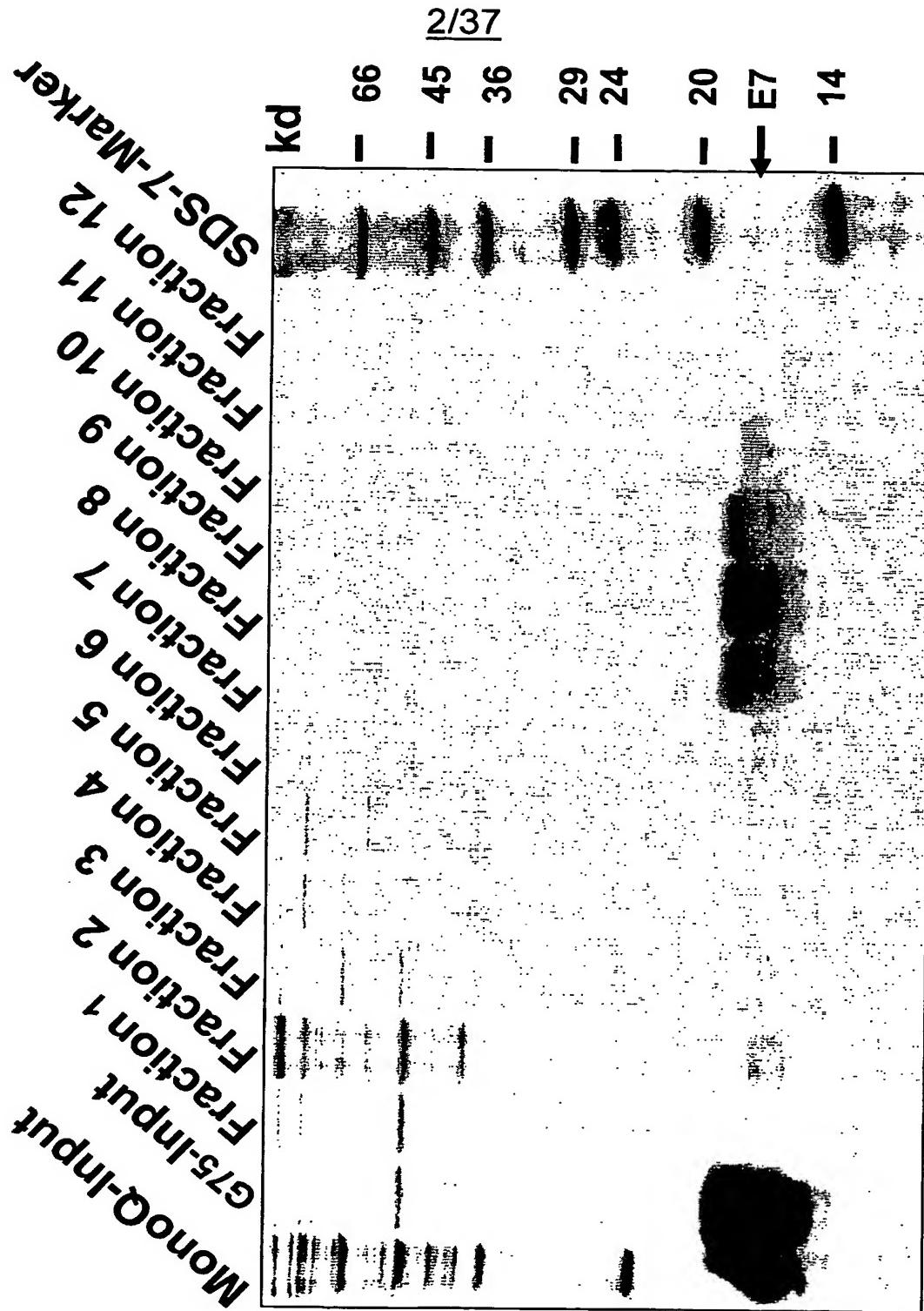


**Figure 1A: Purification of bacterially produced E7 via MonoQ-column
15% PAGE**



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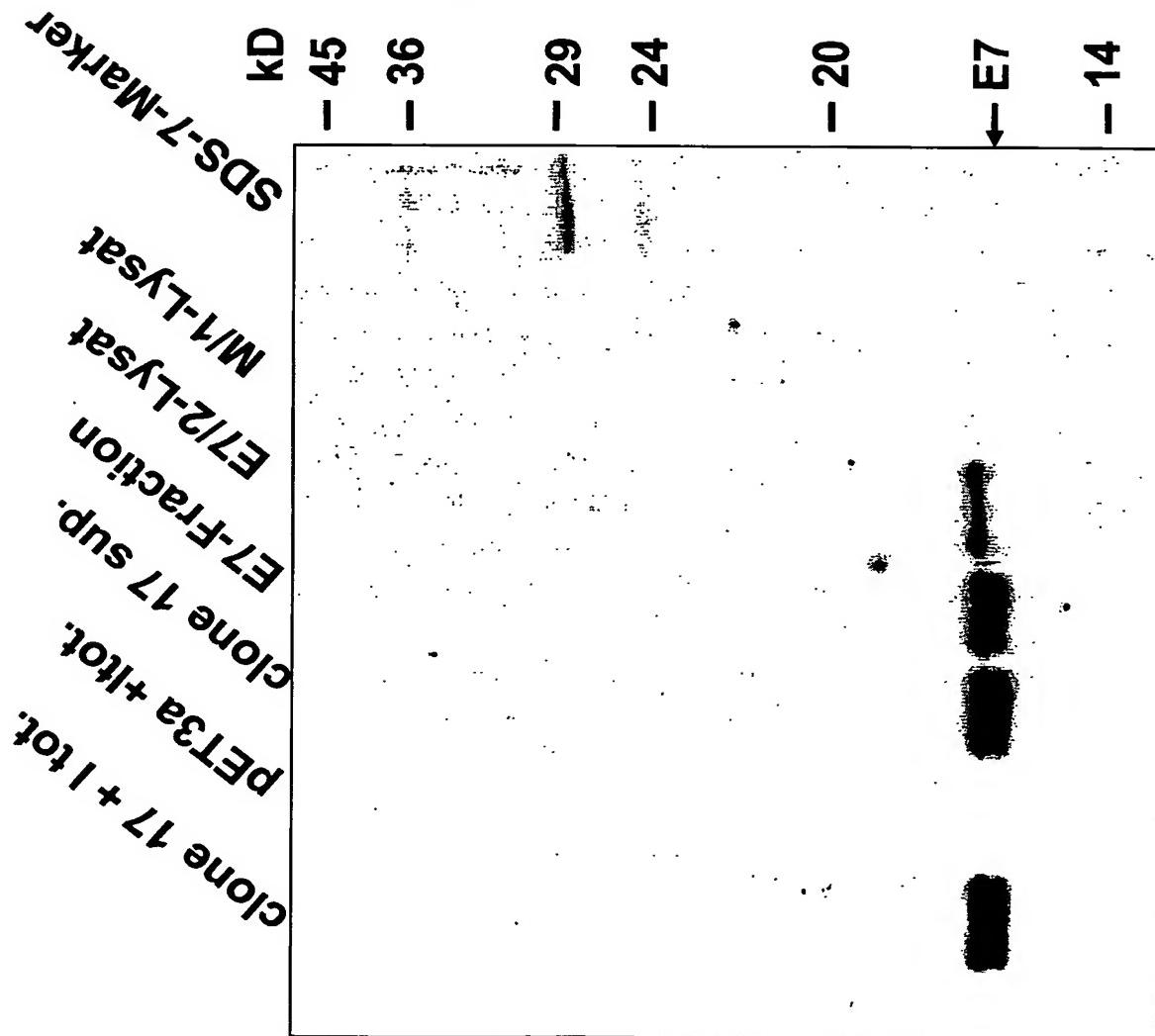
Figure 1B: G75 – Gelfiltration, 15% PAGE



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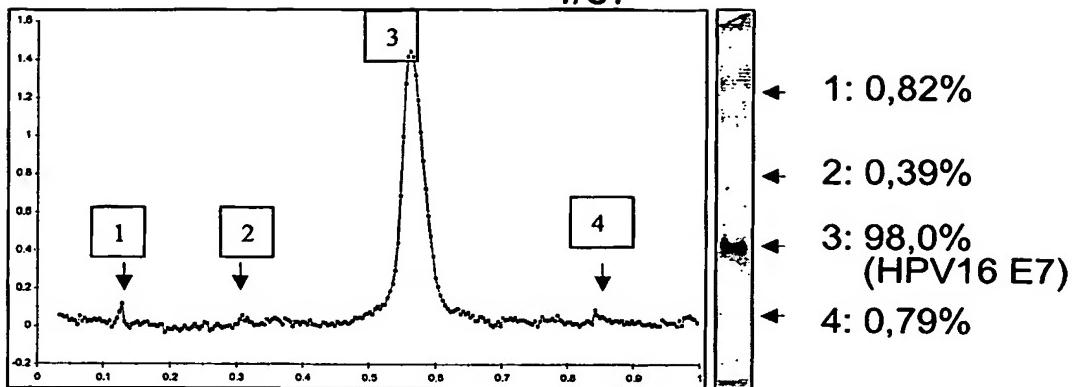
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Figure 1C:
Western Blot,
Santa Cruz (sc 6981)
monoclonal anti-E7
antibody 1:5000

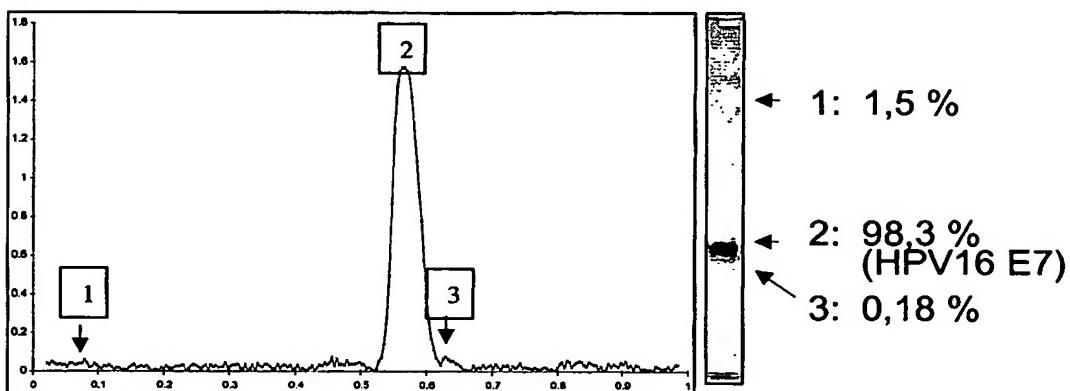


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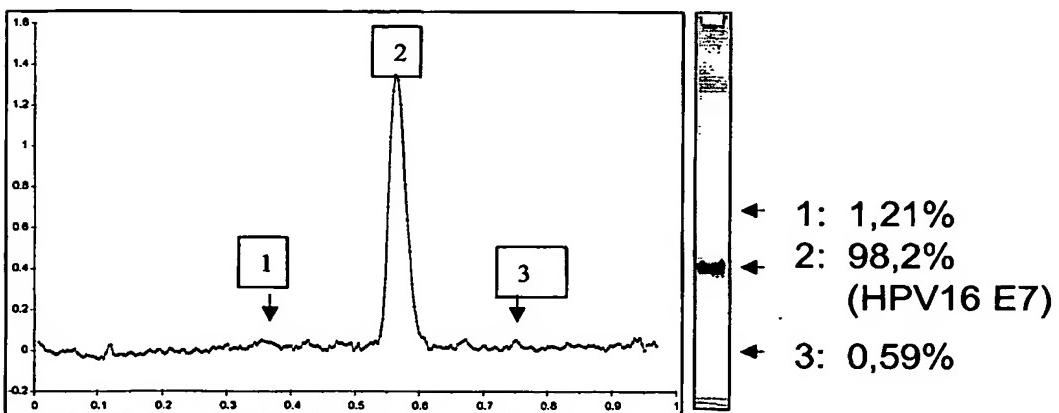
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E7 preparation A



E7 preparation B



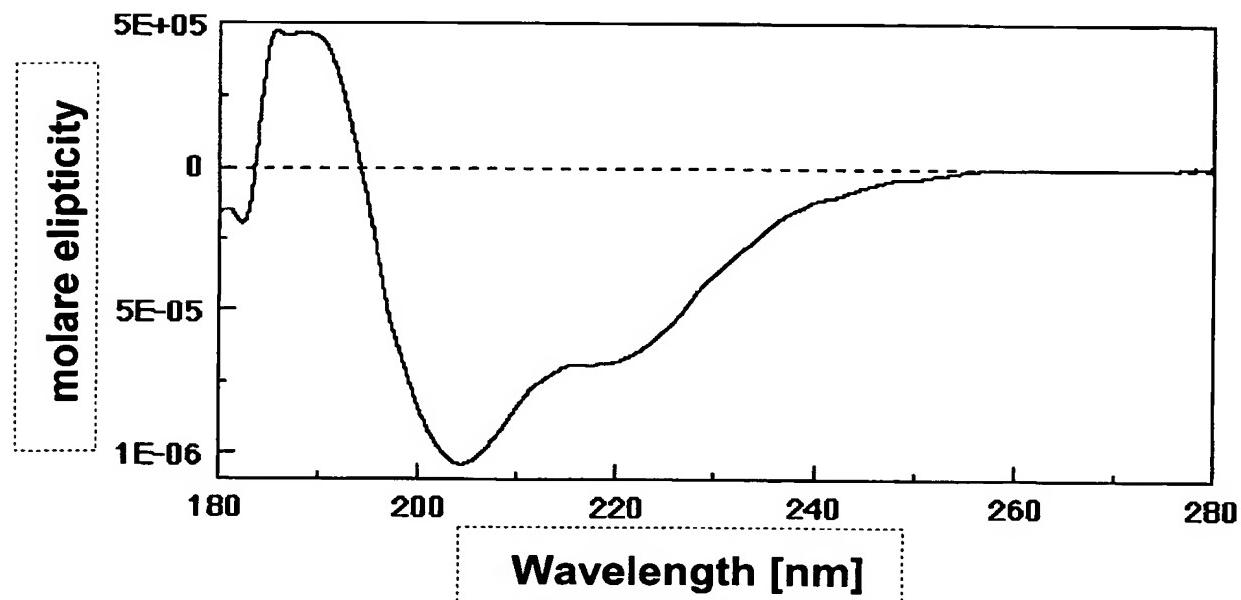
E7 preparation C

Figure 1D

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Figure 1E
Circular dichroism spectroscopy measurements:
Secondary structures in HPV16 E7 protein

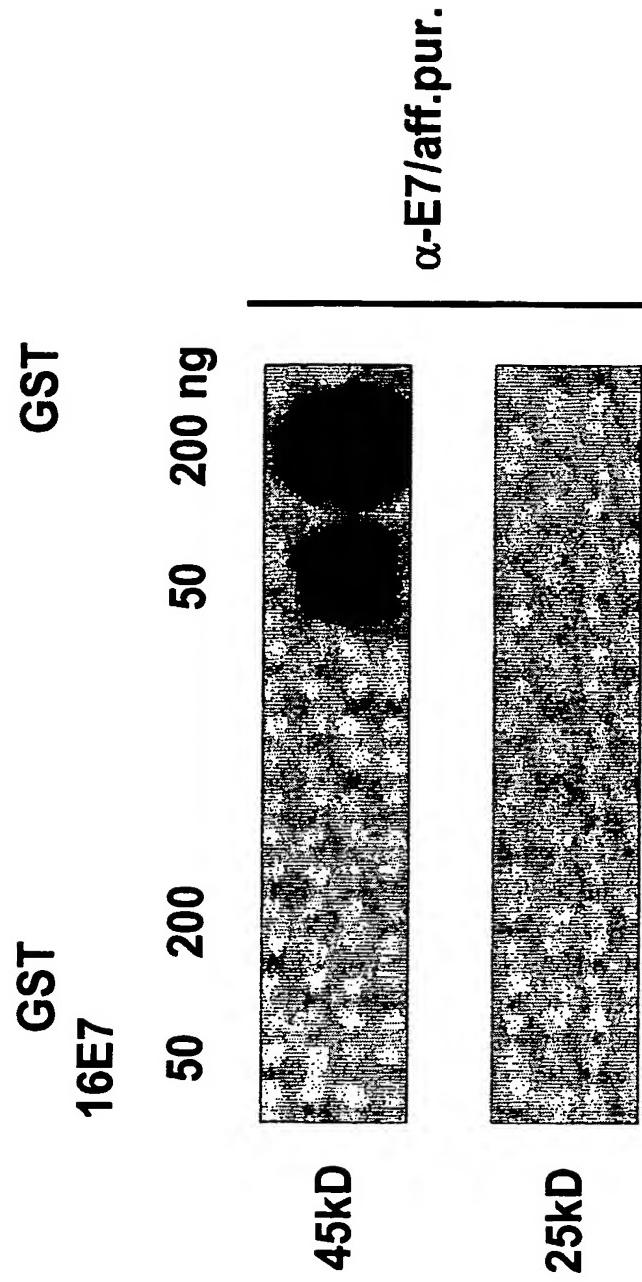


Structure	Approx. %
α -helices	7 - 8 %
β -sheets	45 - 47 %
turn	3 - 5 %
coils	40 - 43 %

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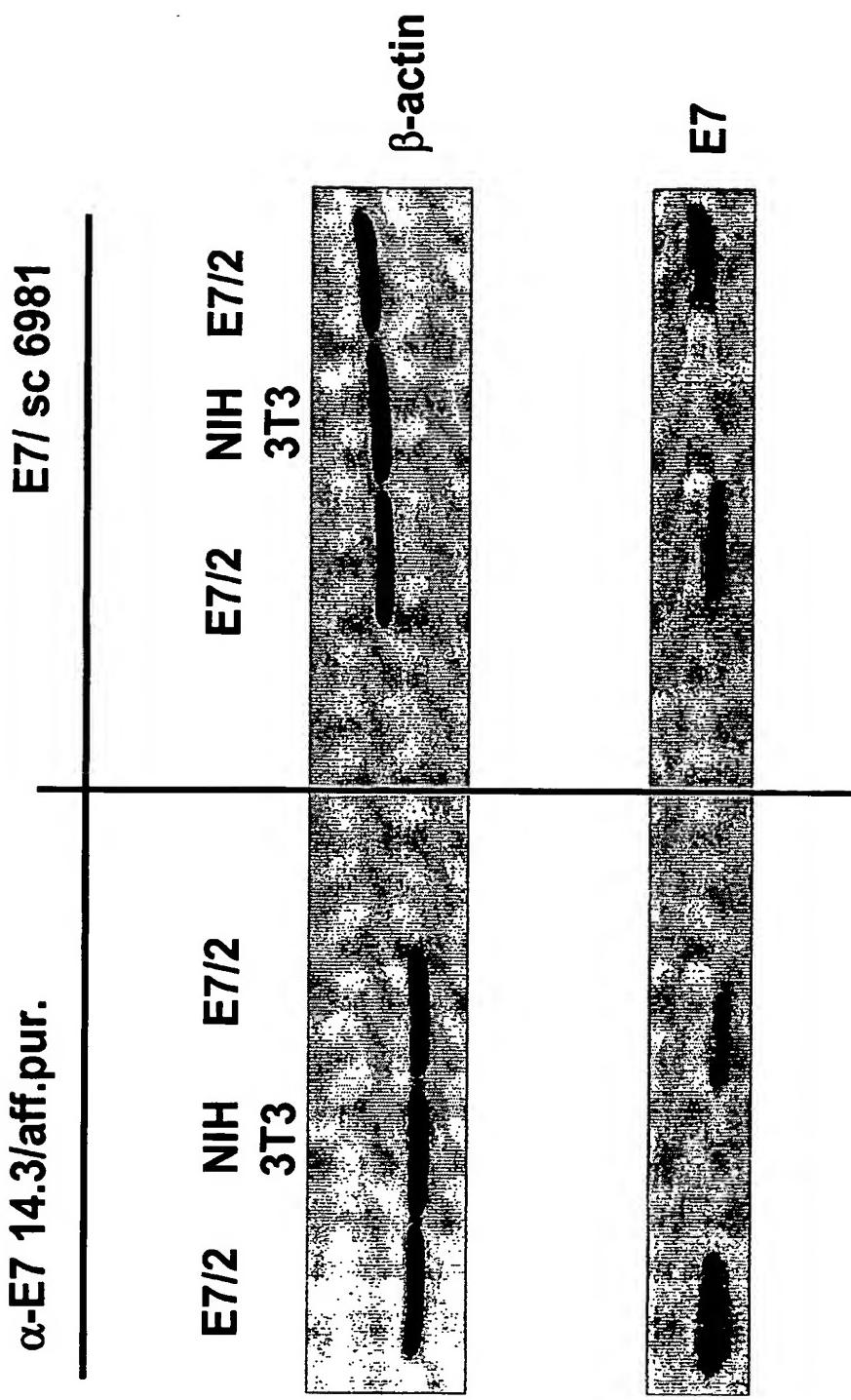
Figure 2A:
Westernblot rabbit anti-HPV-16 E7 antibody (14/3)



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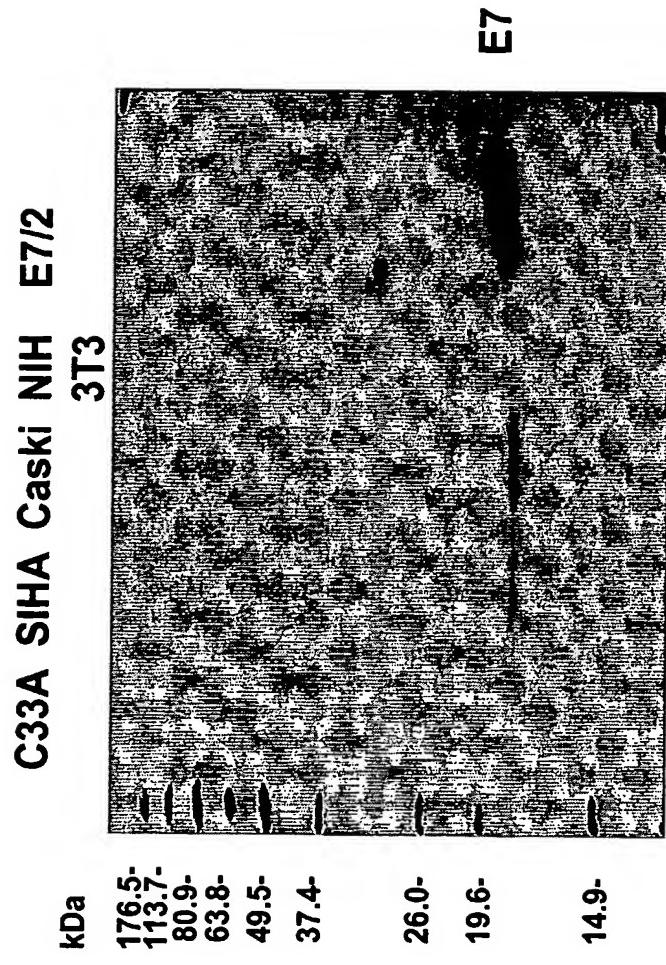
Figure 2B: Westernblot rabbit anti-HPV-16 E7 antibody versus monoclonal anti-HPV-16 E7 antibody Santa Cruz (sc 6981)



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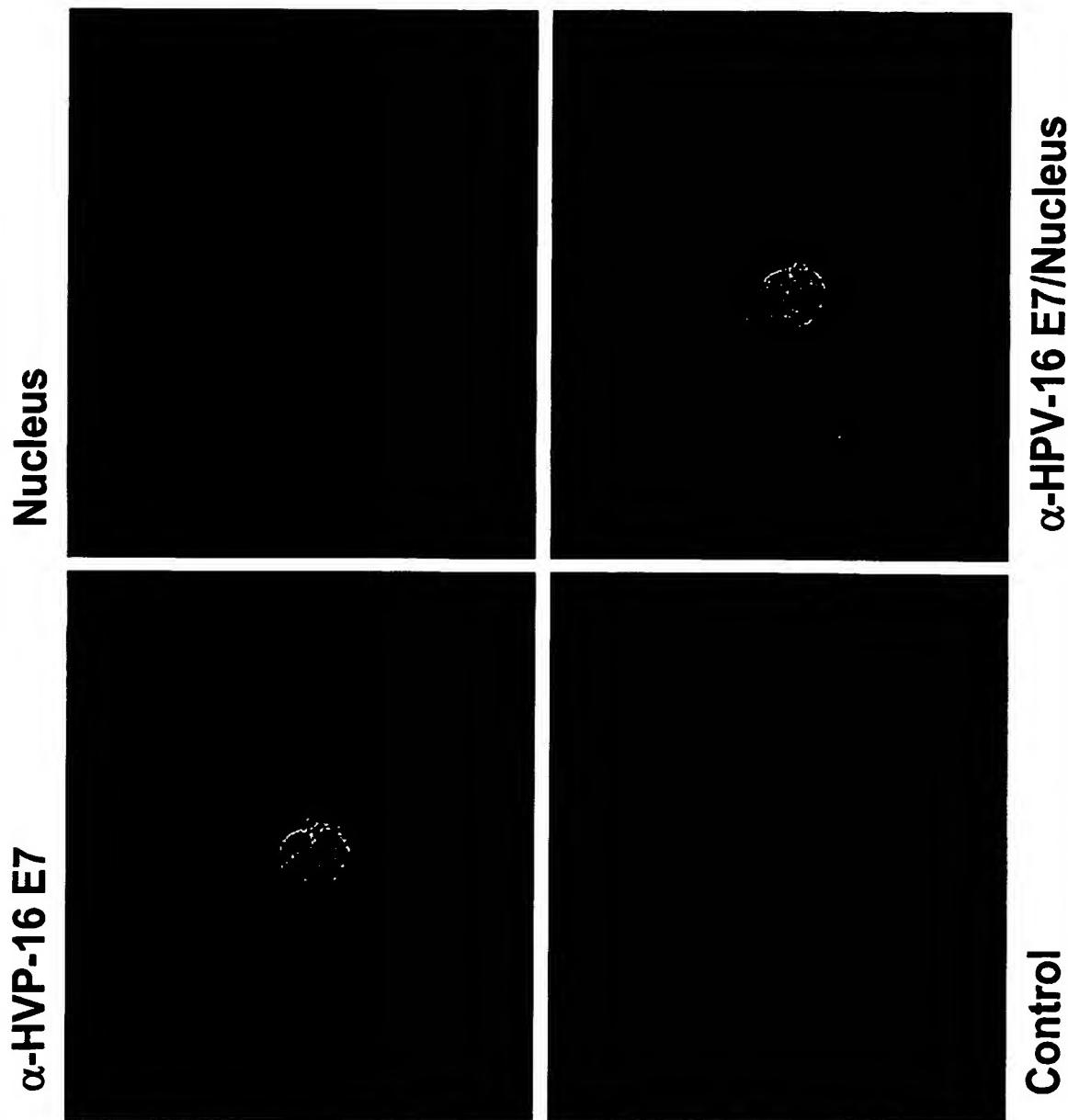
Figure 2C:
Westernblot affinity purified goat anti-HPV-16 E7 antibody,
HPV-16 E7 detection in cell lysates



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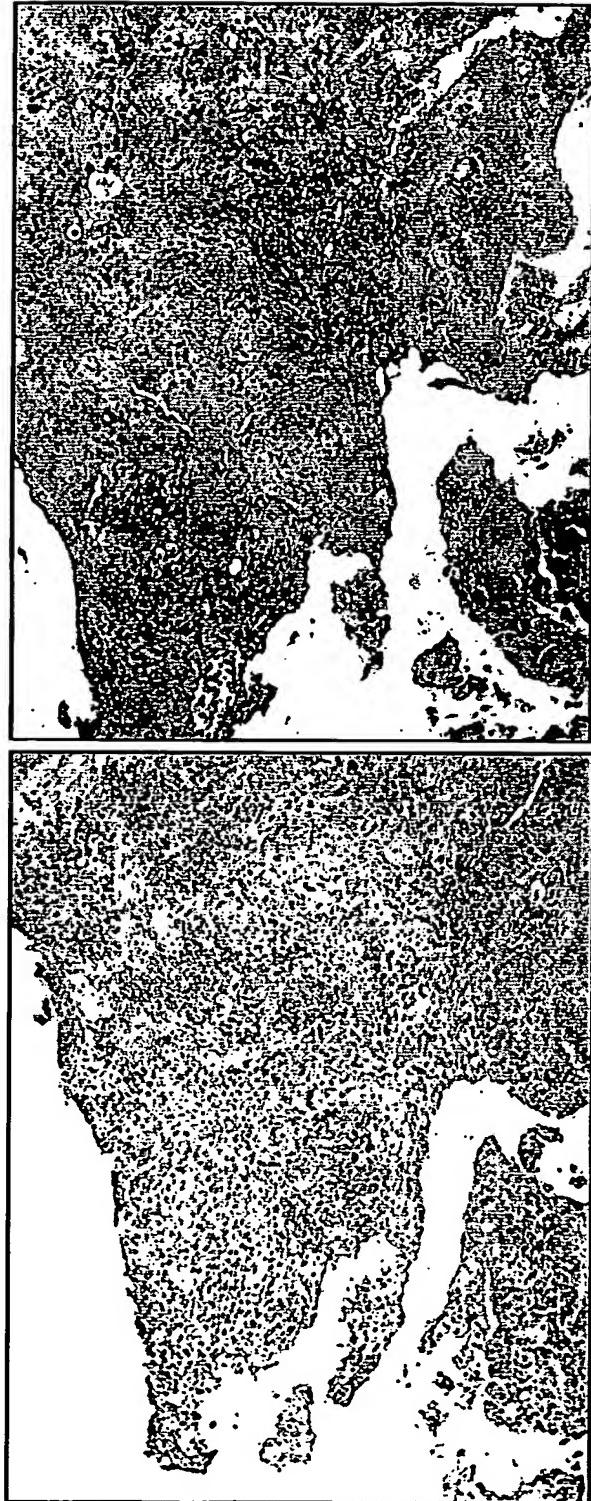
Figure 3
U-2-OS cells transiently transfected with
pX-HPV-16 E7



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Figure 4A
Paraffin section: HPV-16 DNA positive cervical carcinoma
Control Preimmunserum (left), rabbit anti-HPV-16 E7 antibodies



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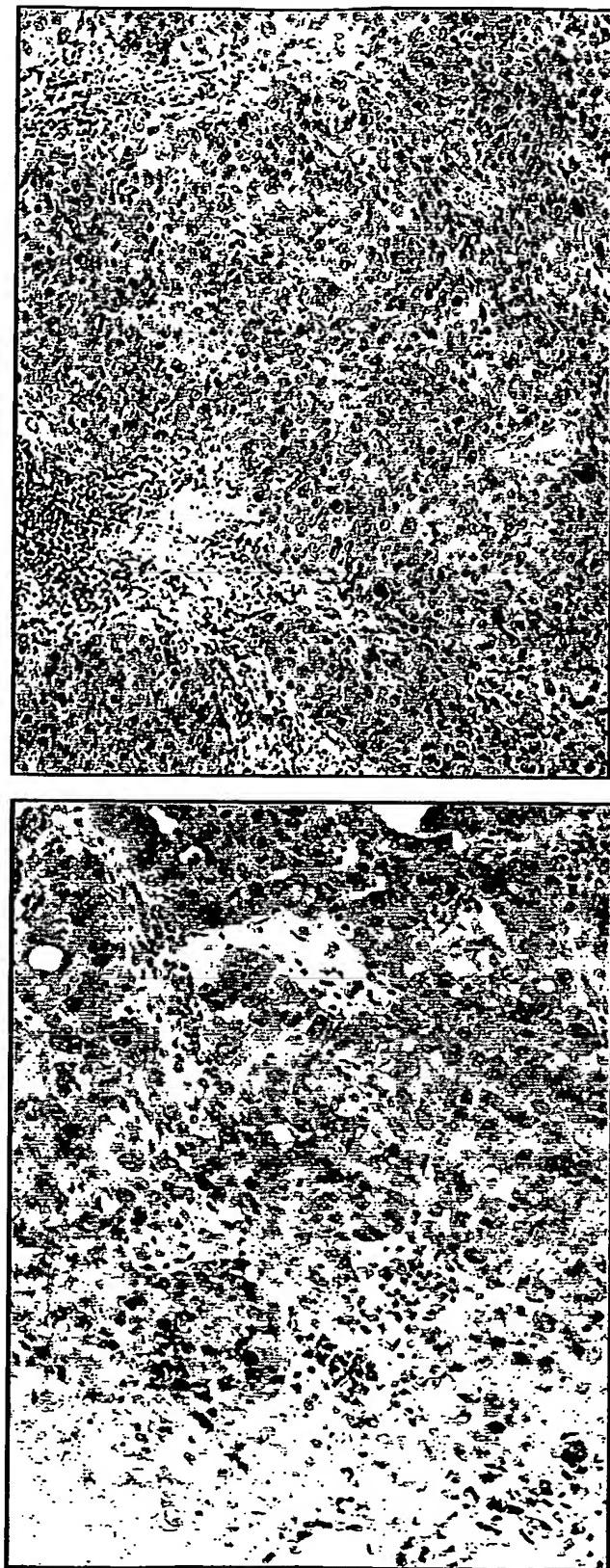
Figure 4B
Paraffin section stained with rabbit anti-HPV-16 E7 antibodies
Negative control normal squamous epithelium (left), no first antibody (right)



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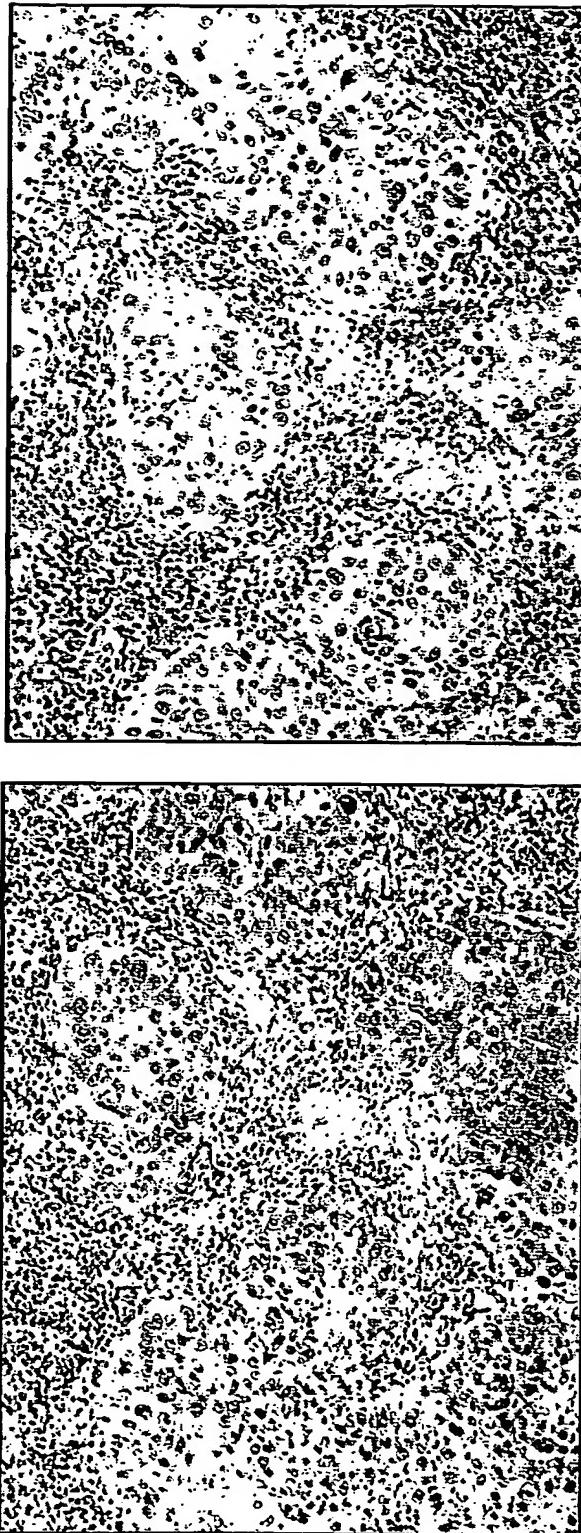
Figure 4C
**Paraffin section: HPV-16 DNA positive cervical carcinoma
stained with rabbit anti-HPV-16 E7 antibodies**



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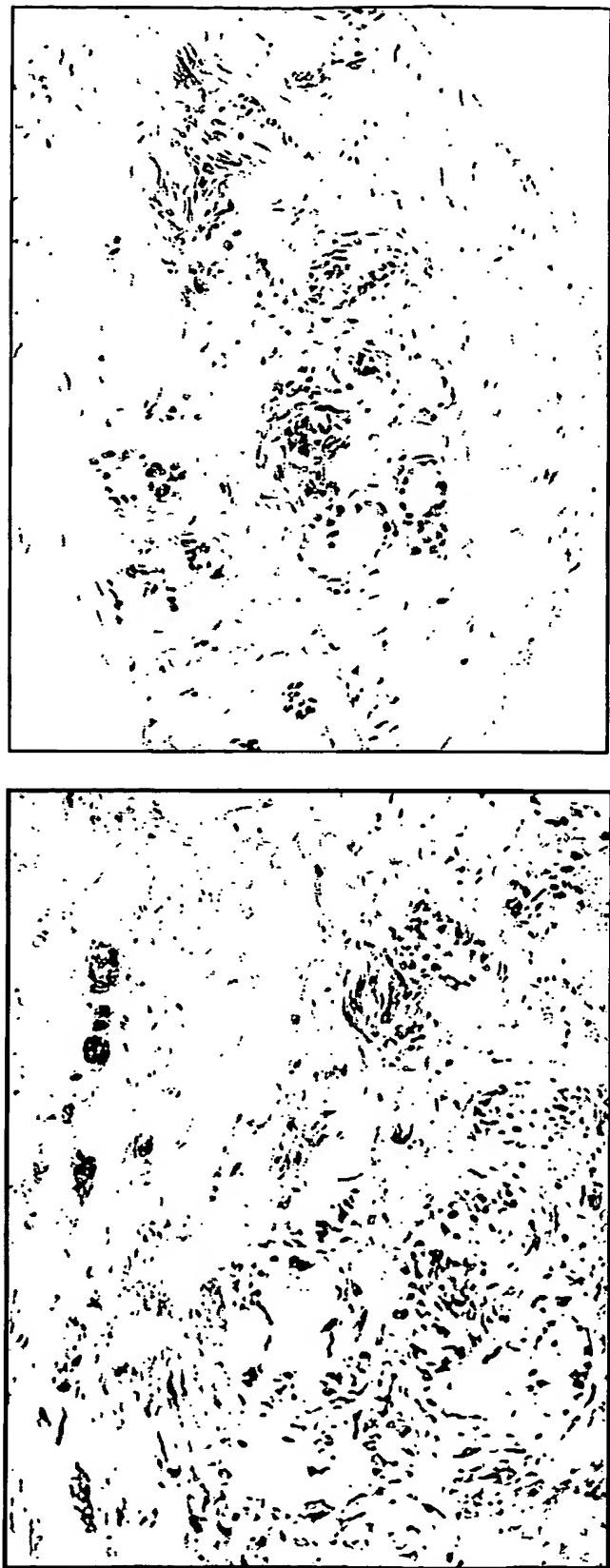
Figure 4D
**Paraffin section: HPV-16 DNA positive cervical carcinoma
rabbit anti-HPV-16 E7 (left) /Competition 1 μ g/ μ l 16 E7 (right)**



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Figure 5
E7-positive prostate carcinoma (left)
E7-negative prostate carcinoma (right)



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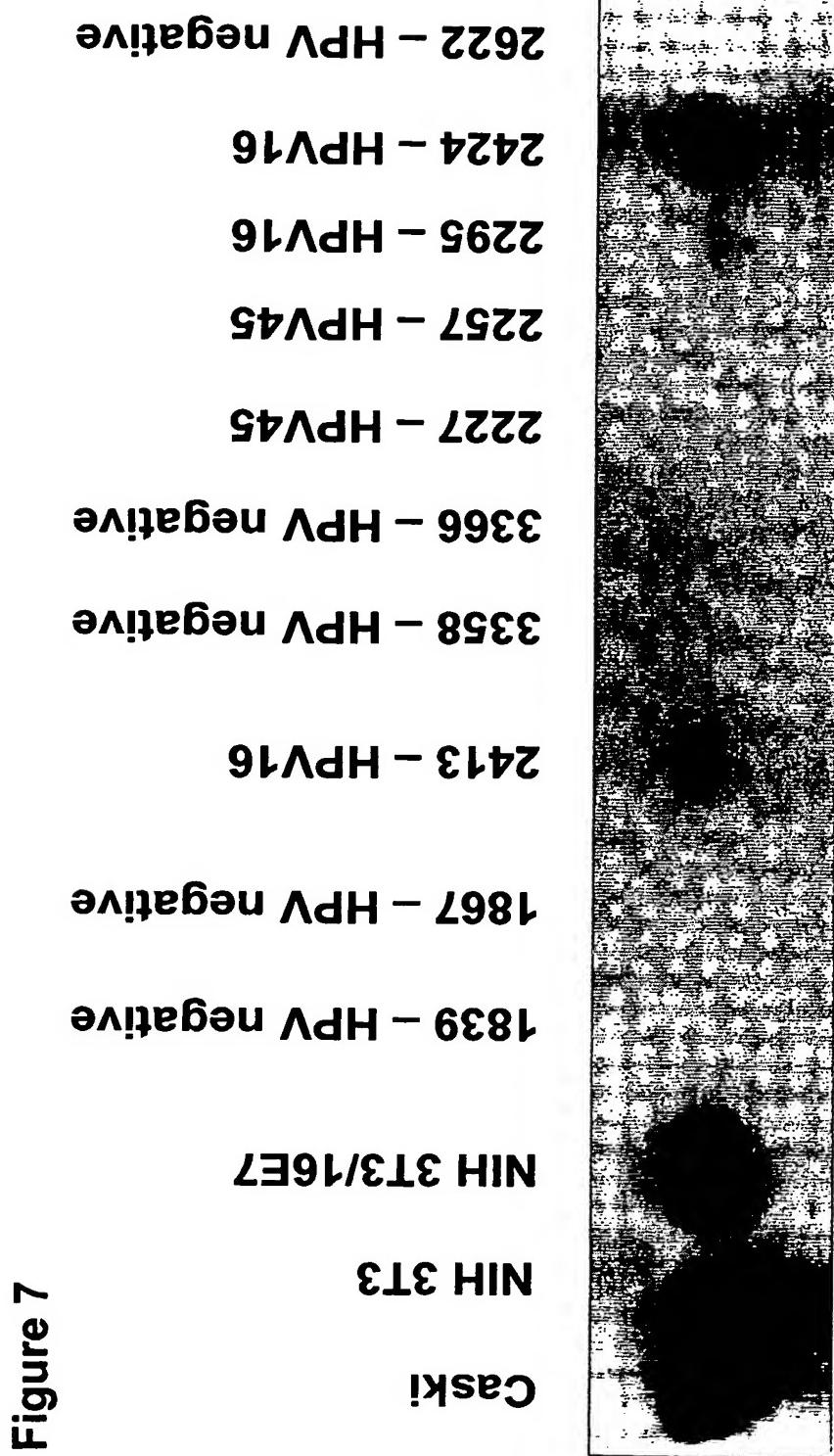
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Figure 6

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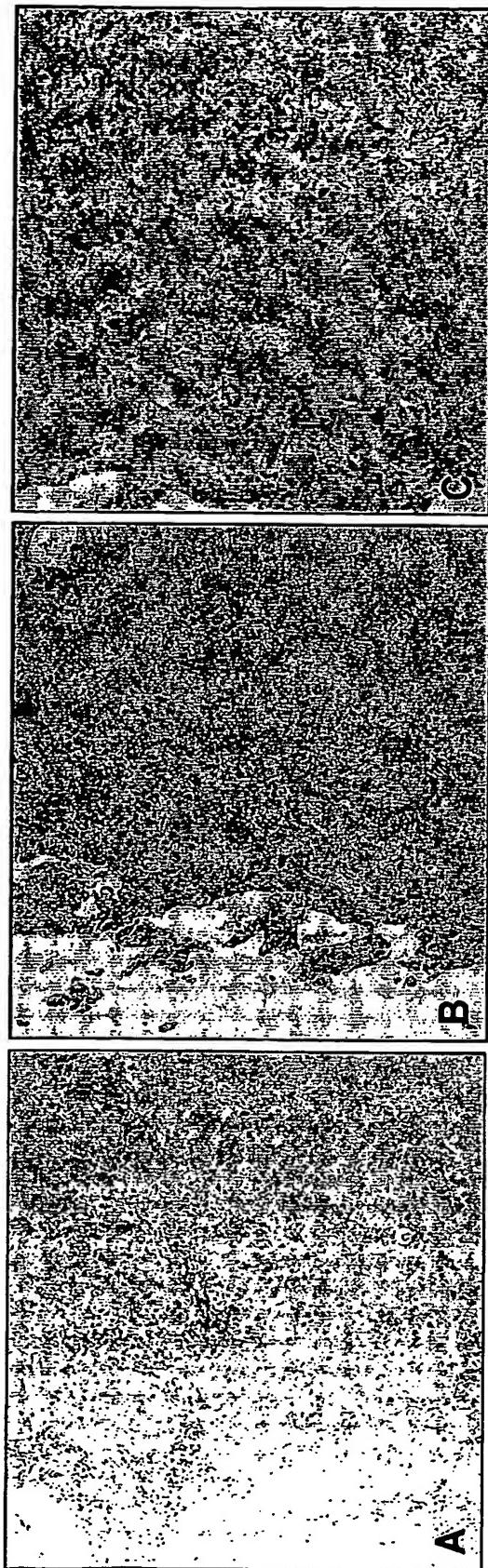
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Figure 8
Immunohistochemical staining of HPV-16 E7 protein in paraffin embedded cervical carcinoma tissue sections ;
A: anti HPV-16 E7 antibody described herein;
B: monoclonal anti-HPV-16 E7 antibody Santa Cruz, clone ED17;
C: monoclonal anti-HPV-16 E7 antibody Zymed, clone 8C9X.

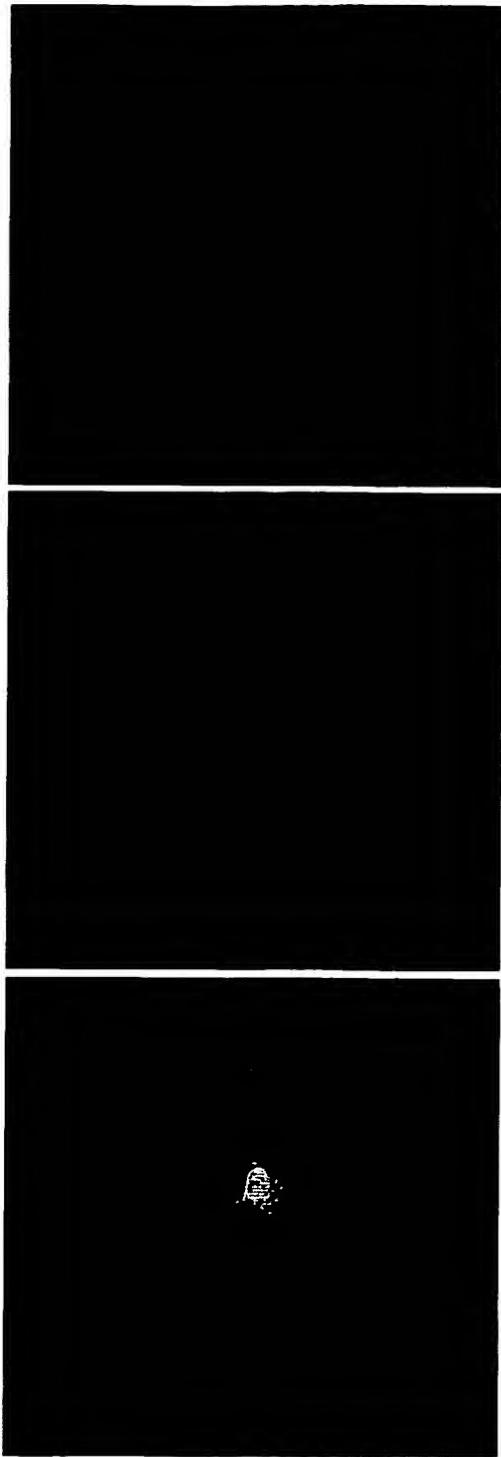


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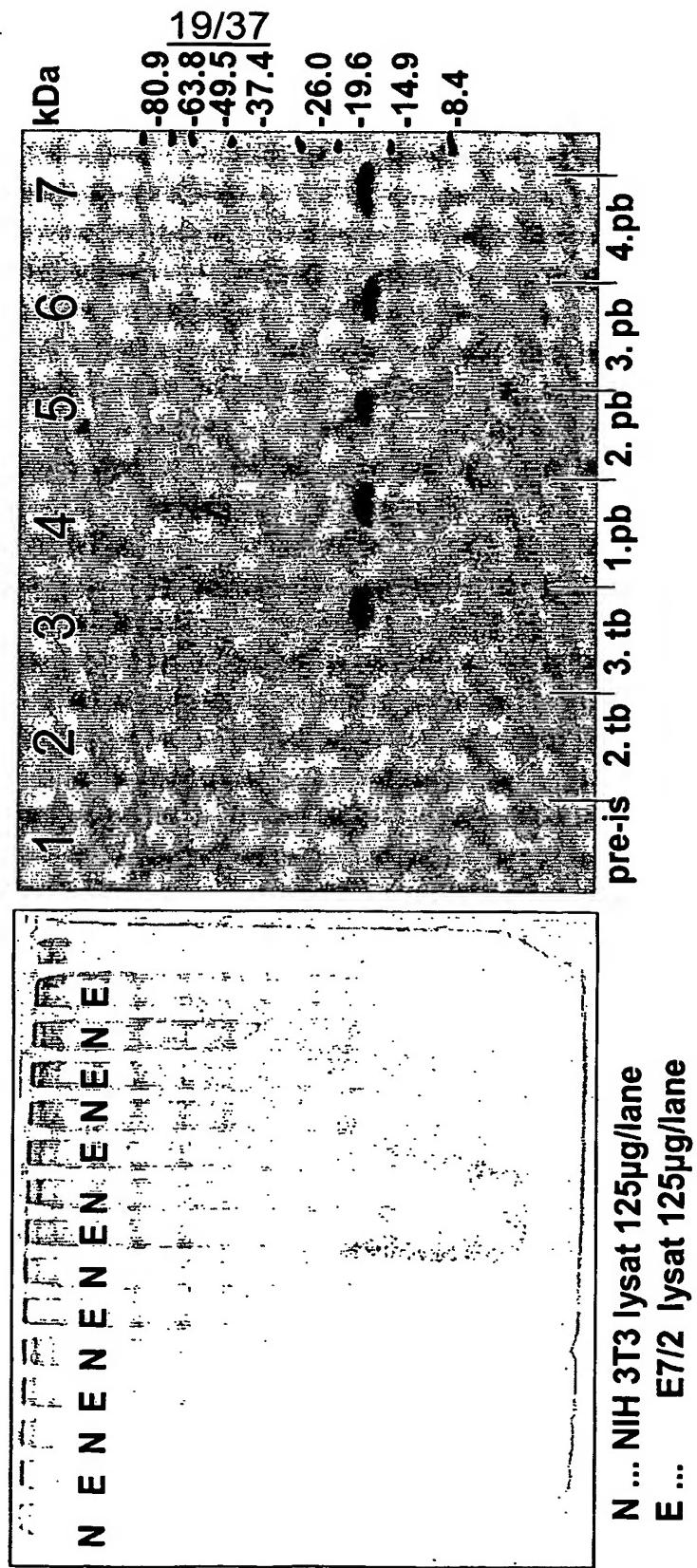
Figure 9
U-2-OS cells transiently transfected with pX-HPV-16 E7

**rabbit anti-HVP-16 E7 anti-HVP-16 E7 Santa
ab described herein Cruz, clone ED17 Zymed, clone 8C9X**



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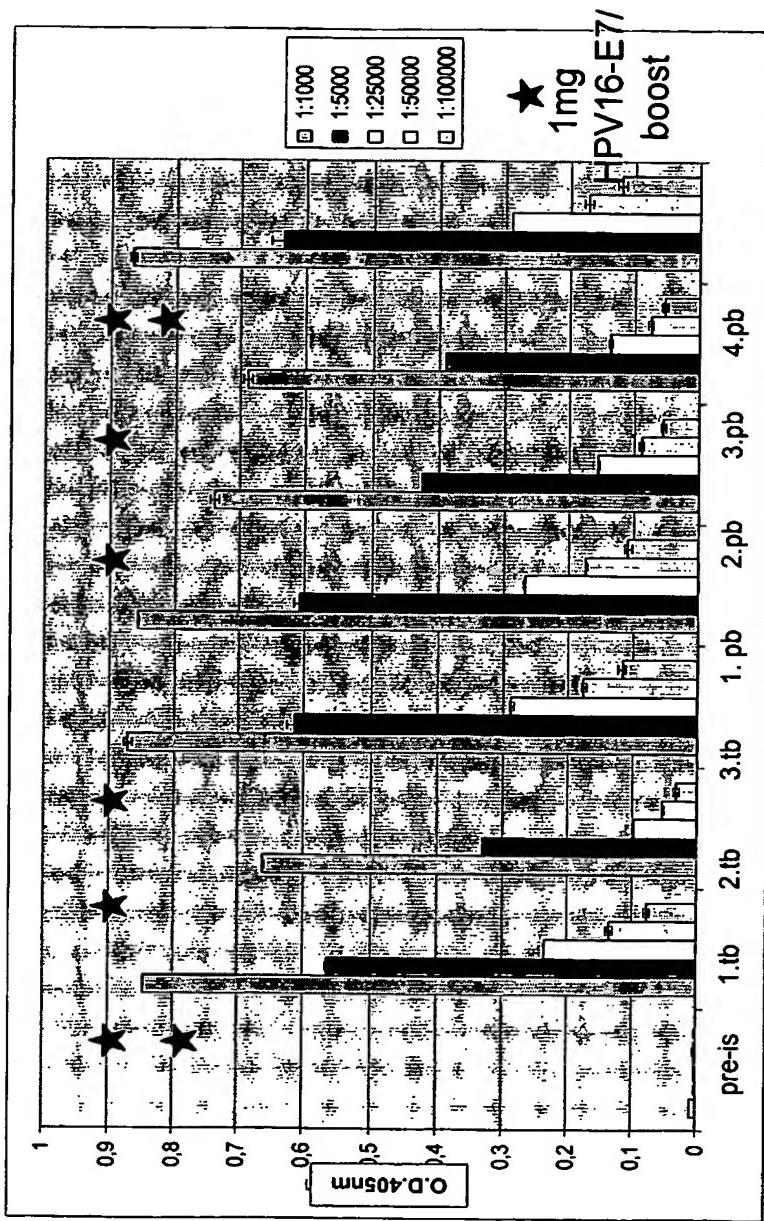
Figure 10 :
Western-blot as quality control of goat-HPV16-E7 antiserum



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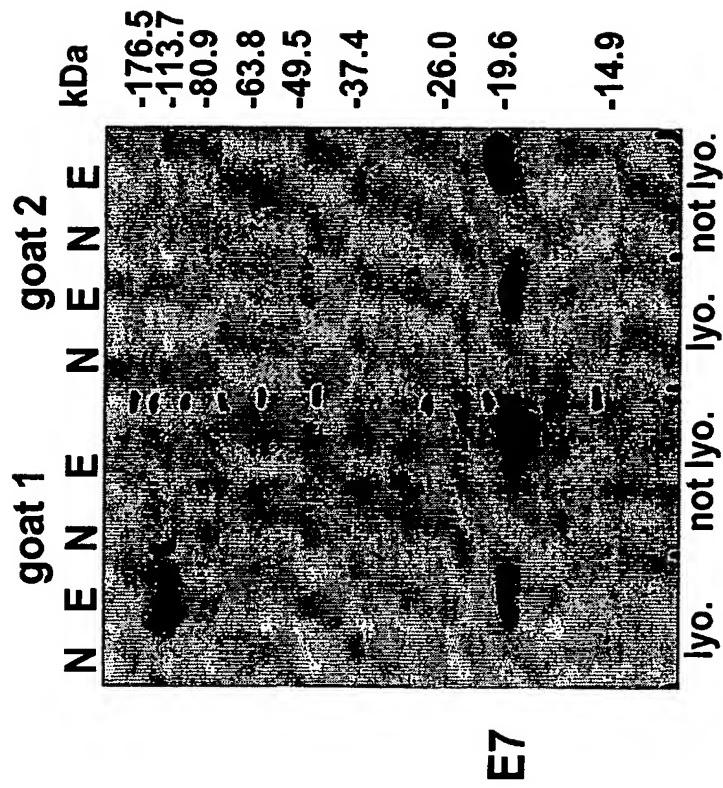
Figure 11 :
Direct ELISA to determine the titer of goat HPV16-E7 antiserum



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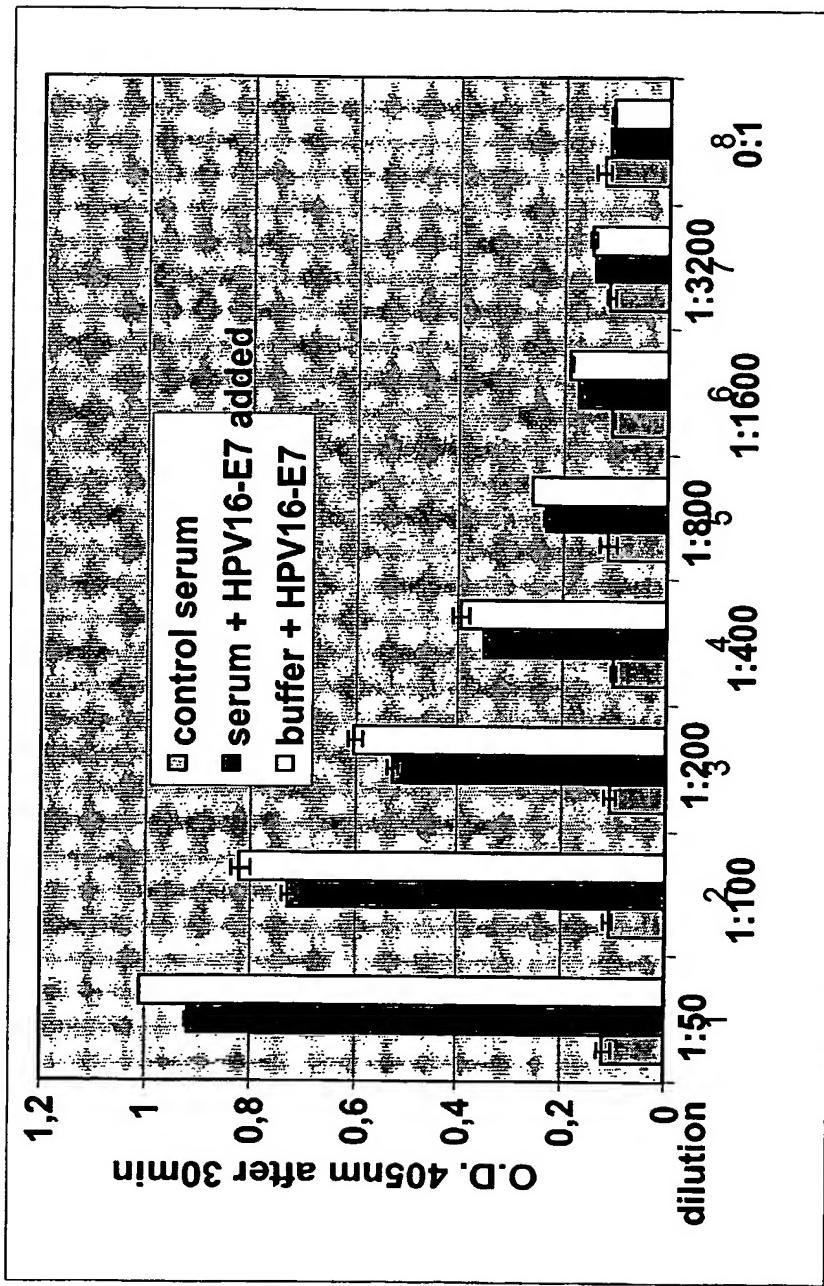
Figure 12:
**Western-blots as quality control for purified, reconstituted
anti HPV16-E7 antibody from 2 goats**



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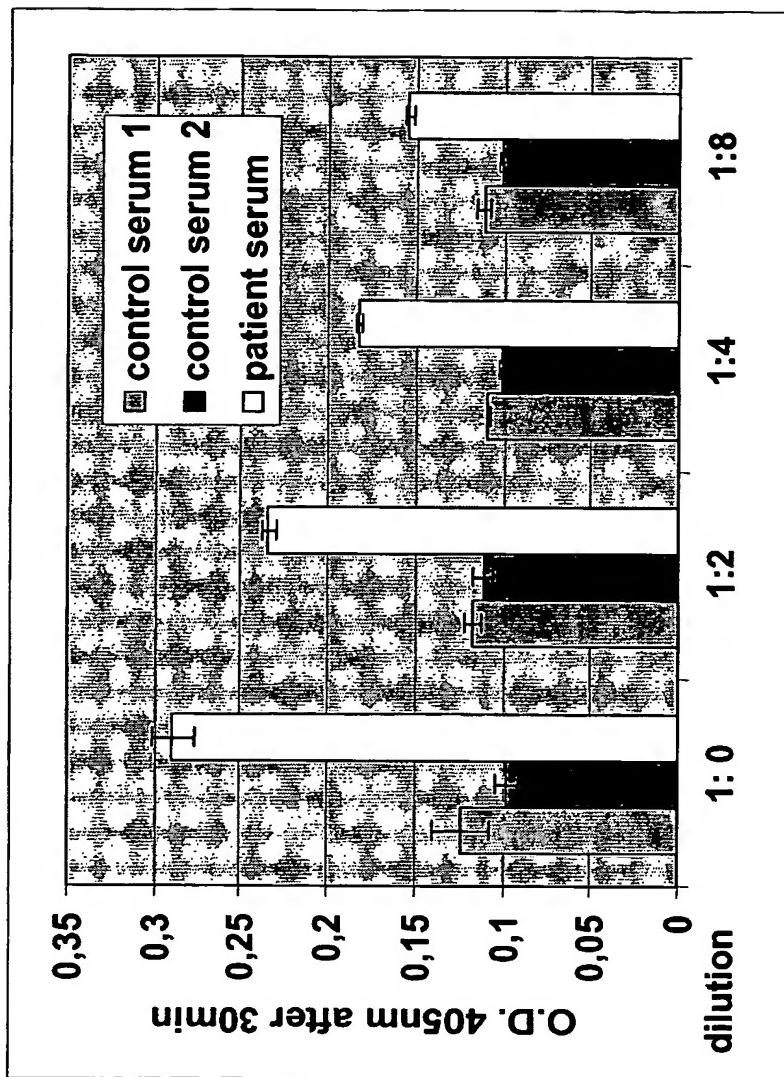
Figure 13 :
Direct sandwich ELISA to detect HPV16-E7 in serum samples



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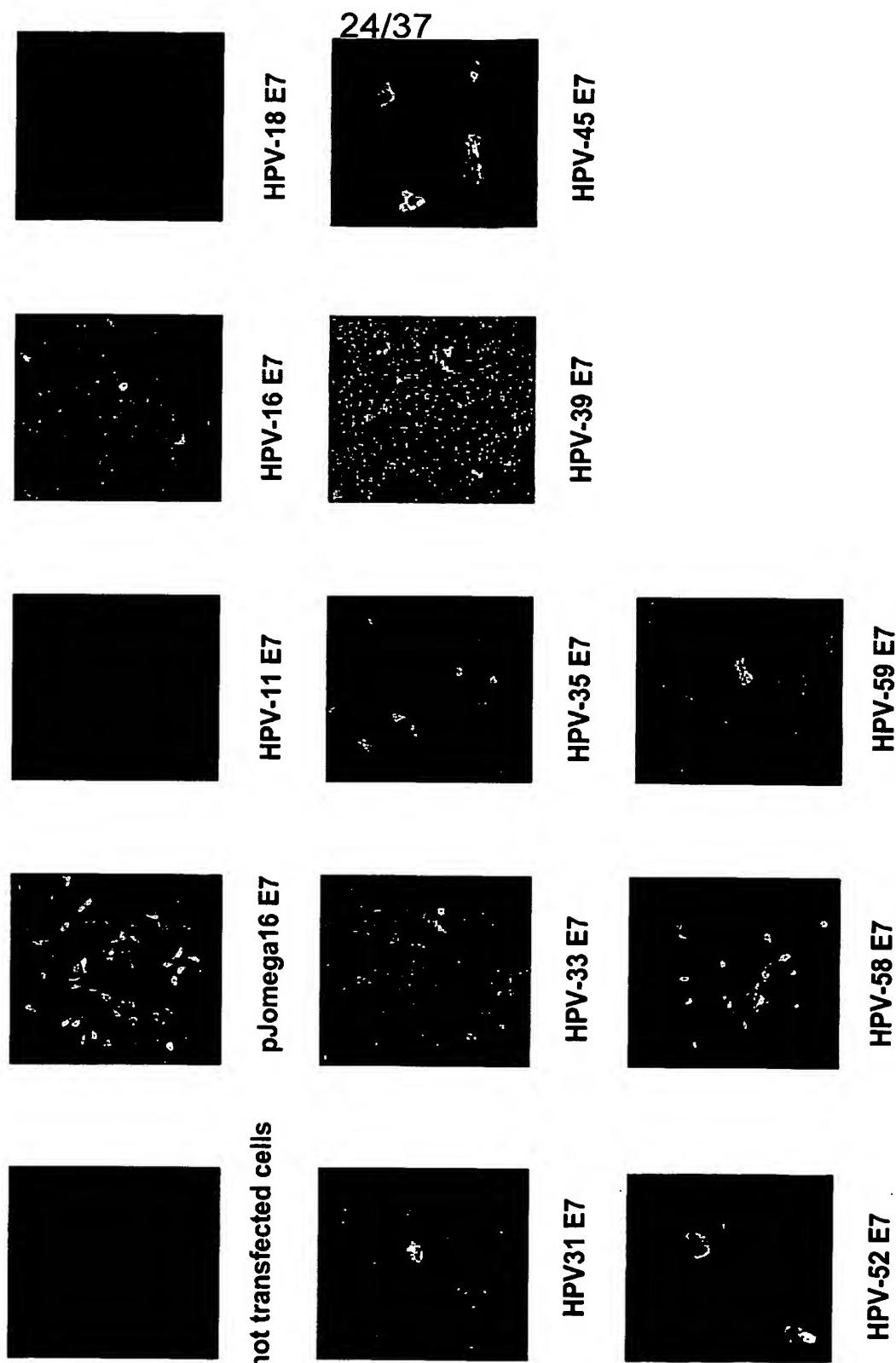
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Figure 14 :
Direct sandwich ELISA to detect HPV16-E7 in patient serum samples



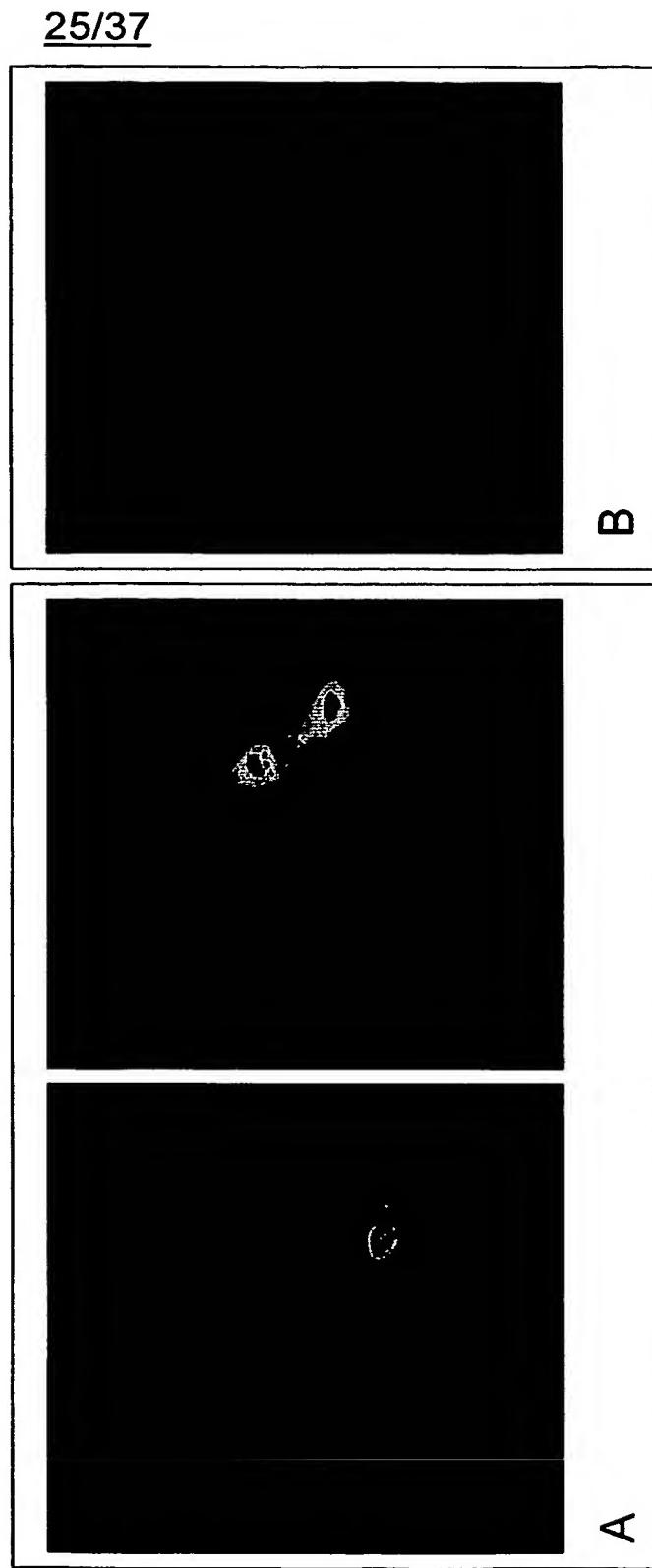
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Figure 15 : Immunofluorescence detection of different high risk HPV E7 proteins by anti HPV-16 E7 antibodies from goat



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Figure 16
**Competition of specific antibody activity from affinity purified
Amylonon goat anti HPV16-E7 pAb:**



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Figure 17:

Purification of recombinant HPV18-E7, expressed in *E.coli*; all SDS-PAGE 12.5% under reducing conditions

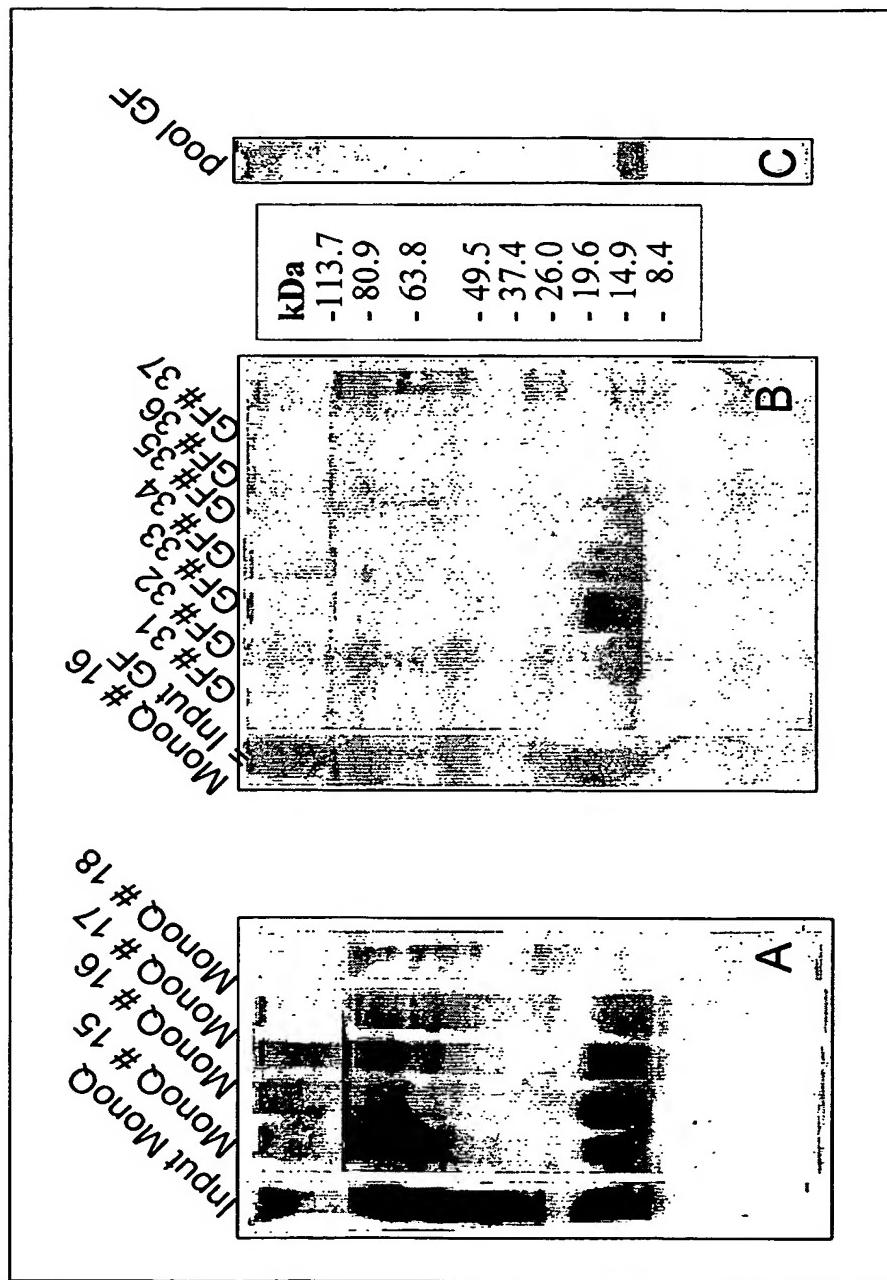
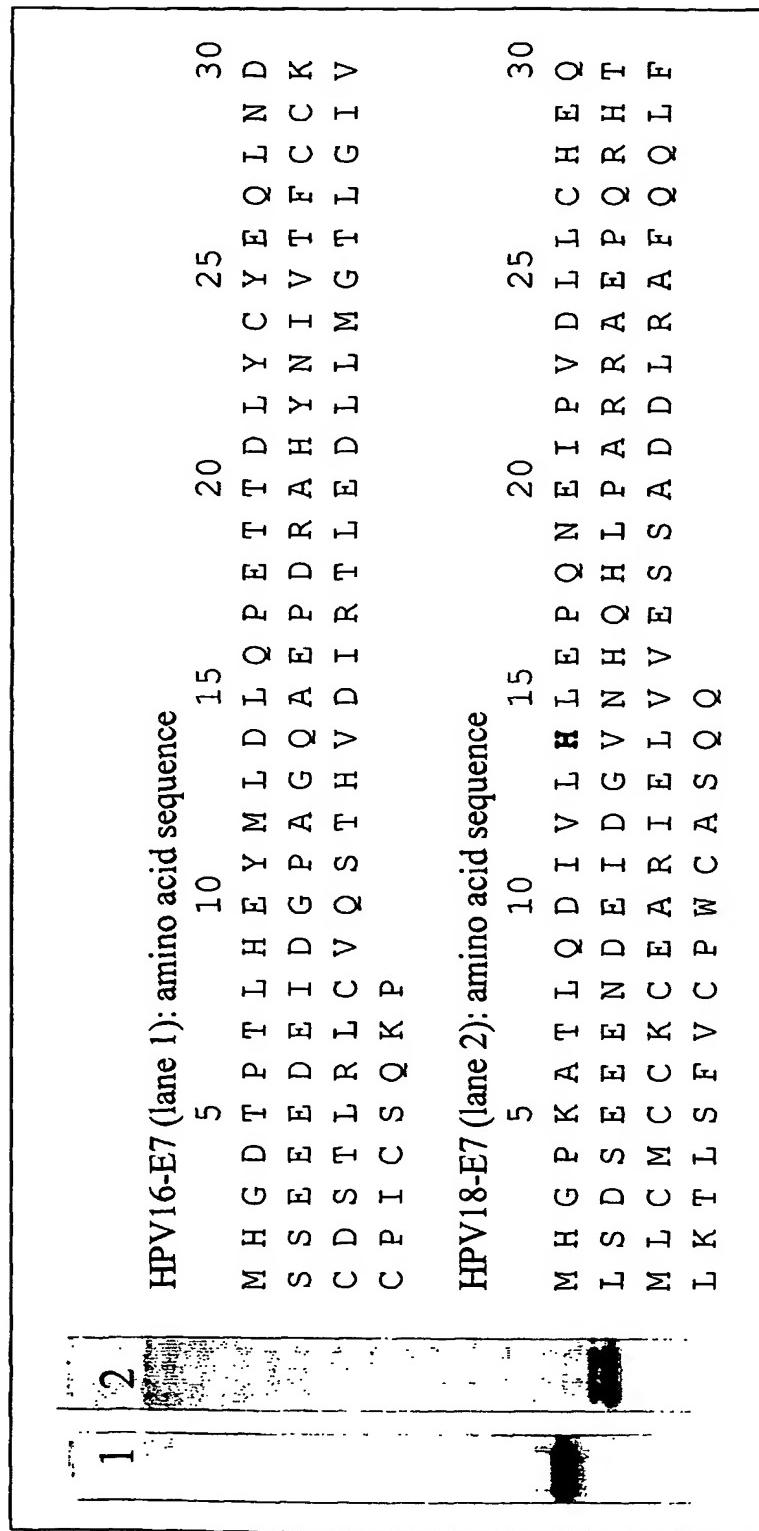
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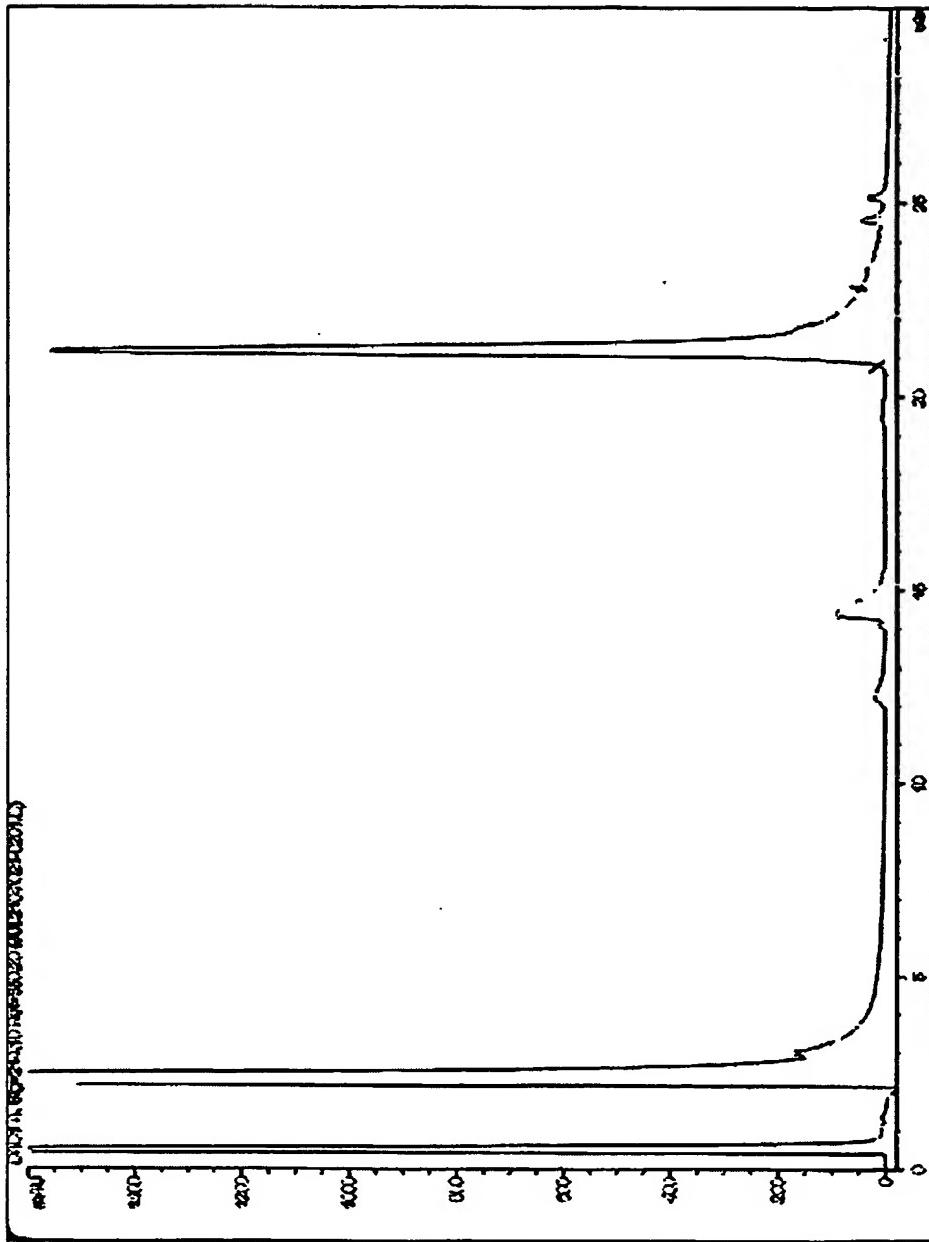
Figure 18A:
Purified HPV16-E7 (lane 1) and HPV18-E7 (lane 2) protein that was used for immunisations.



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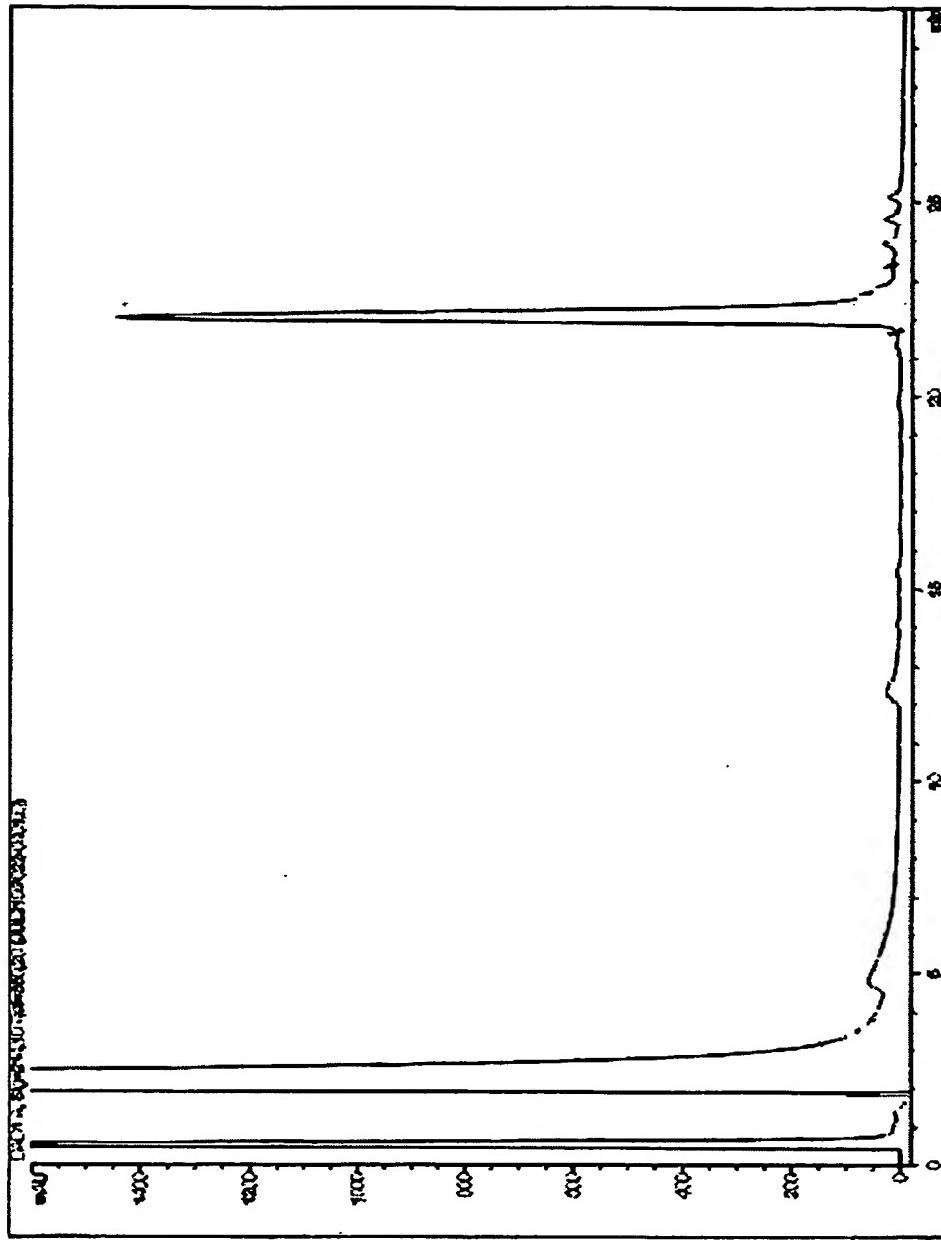
Figure 18B :
**Purified HPV16-E7 protein after reduction and pyridylethylation
prior to tryptic digest.**



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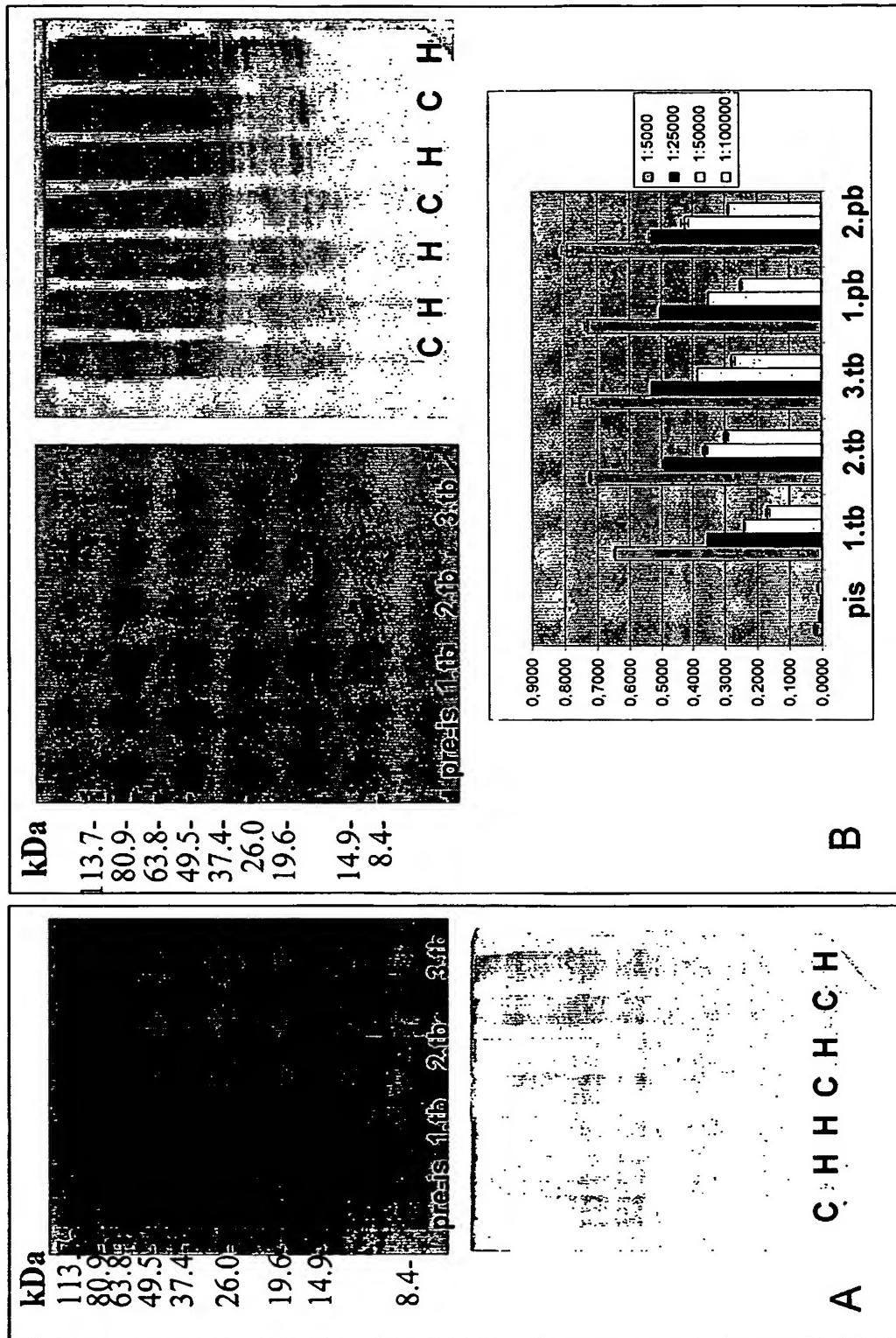
Figure 18C :
Purified HPV18-E7 protein after reduction and pyridylethylation
prior to tryptic digest.



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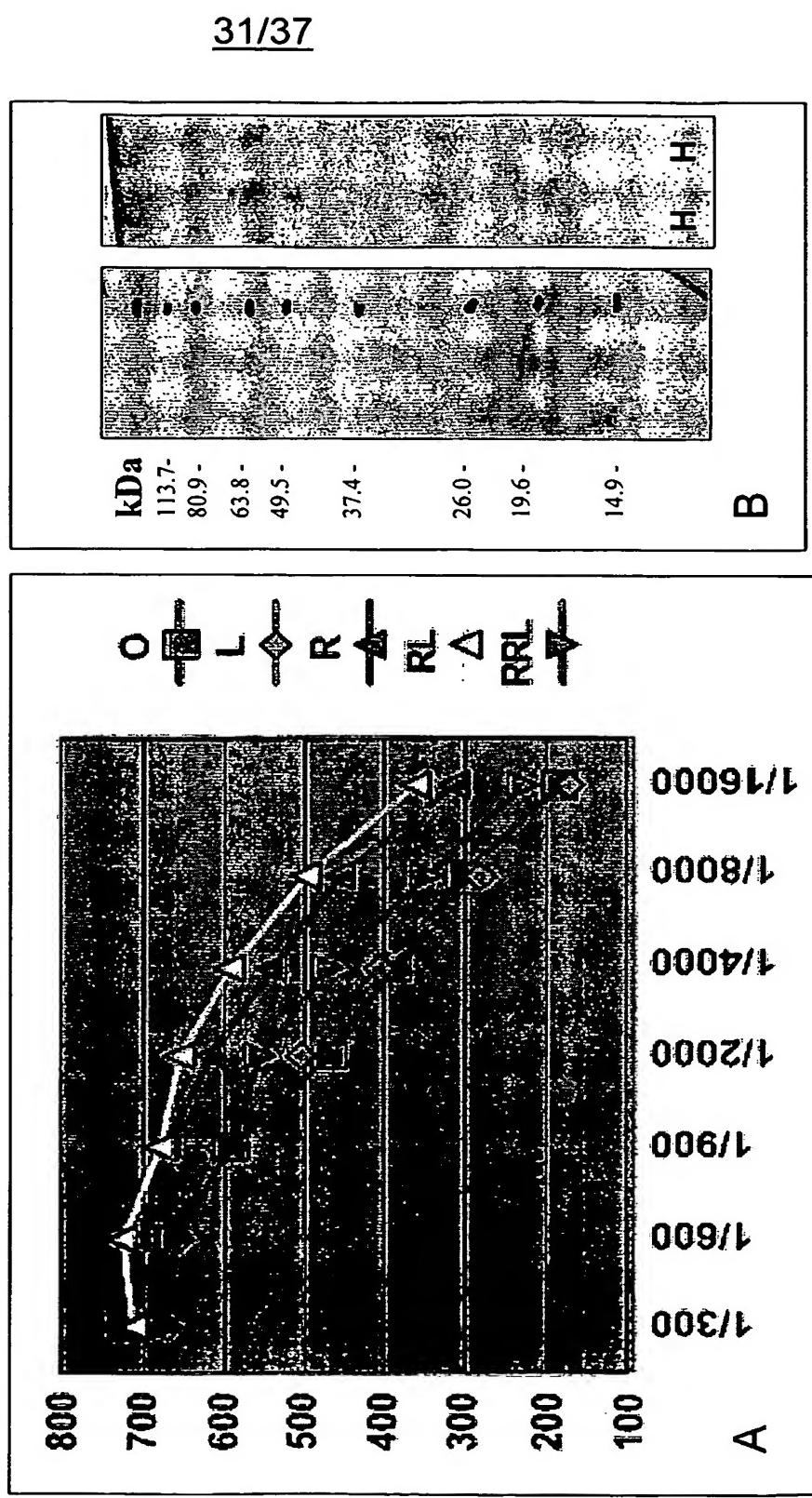
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Figure 19 :
Test of immune responses after immunisation of rabbit (left) and goat (right) with HPV18-E7 protein.



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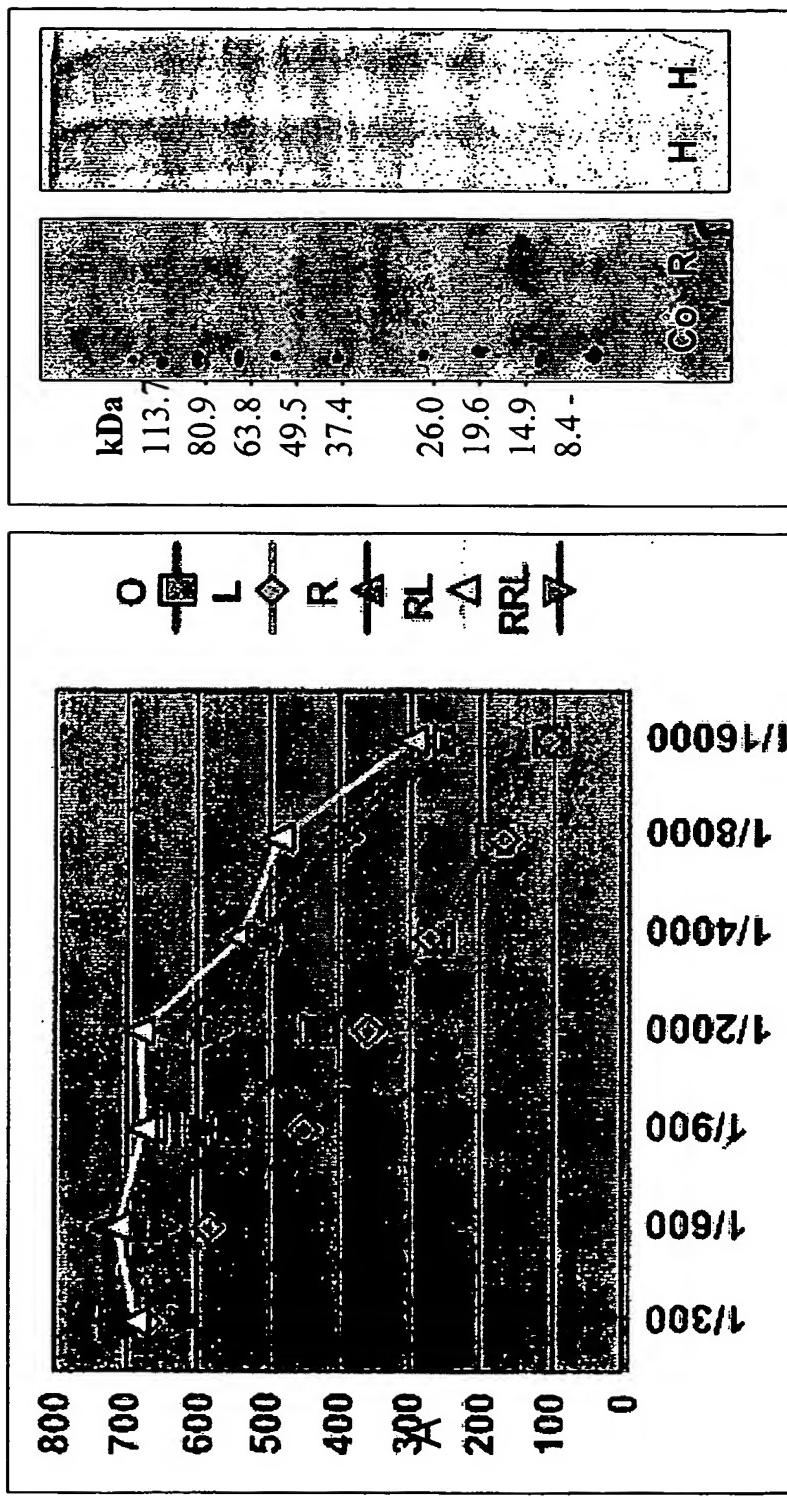
Figure 20A:
ELISA performed with serum from mice (O, L, R, RL, RRL) immunised with
HPV16-E7 protein.



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Figure 20B:
**ELISA performed with serum from mice (O, L, R, RL, RRL) immunised with
HPV18-E7 protein.**

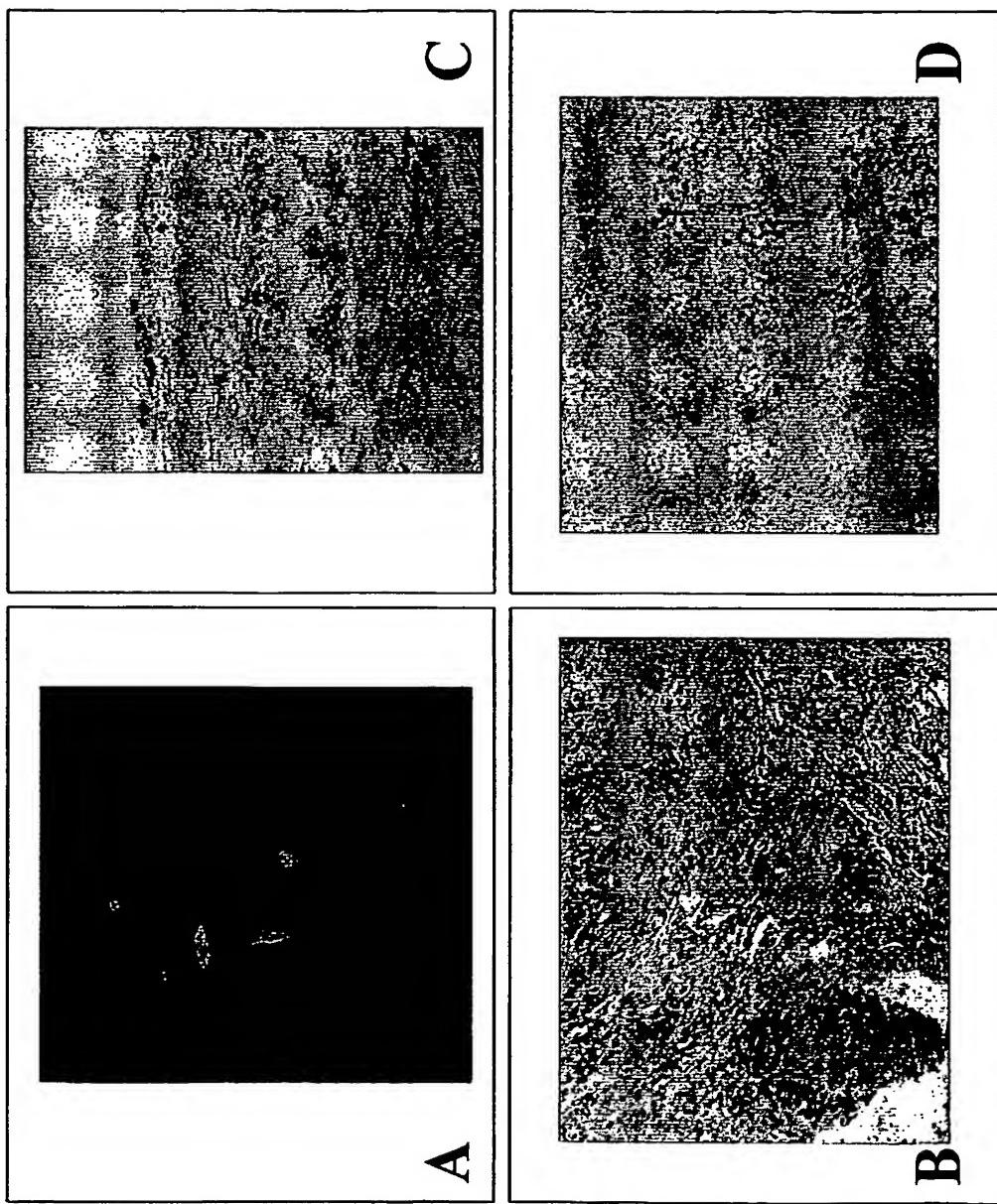
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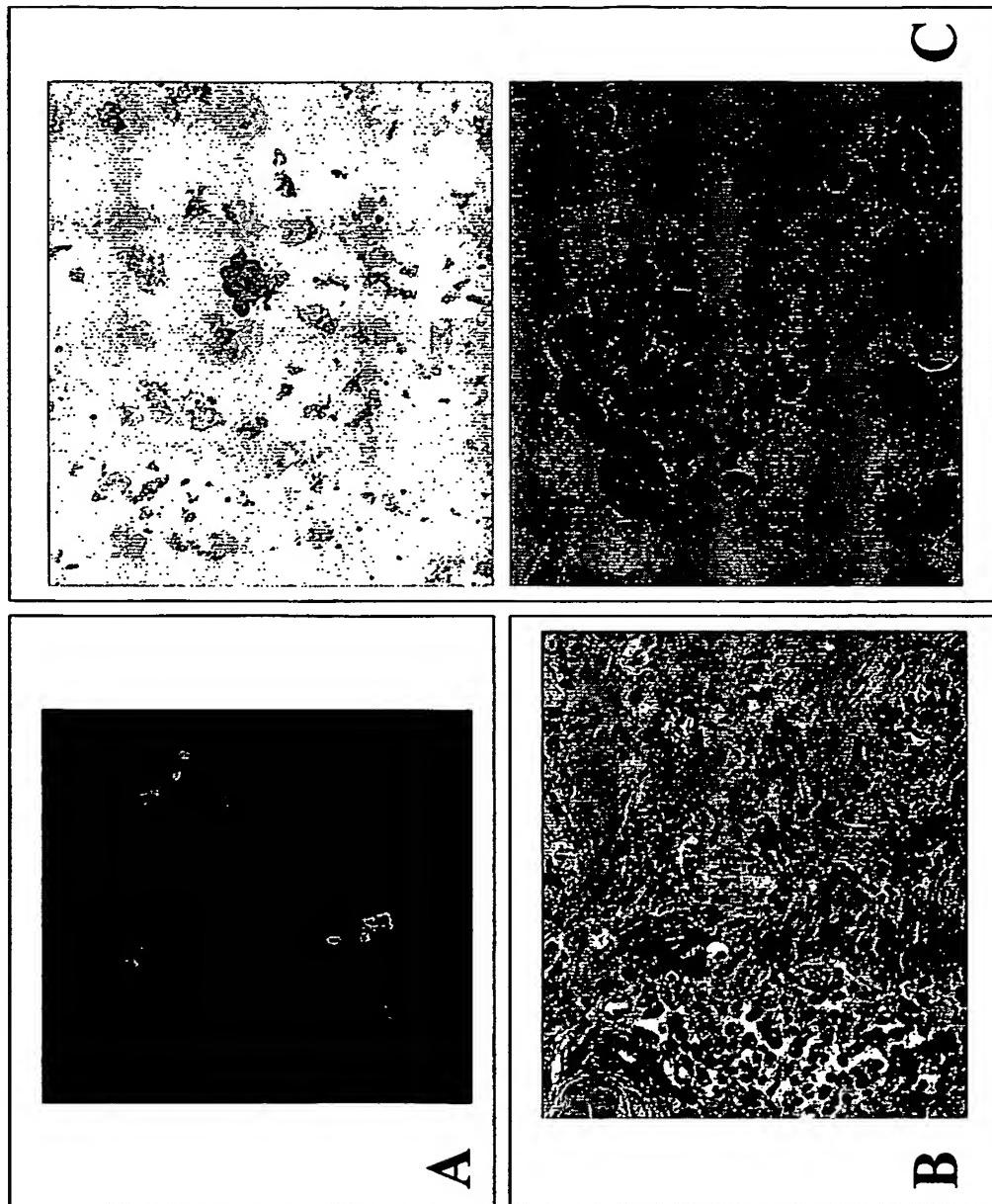
Figure 21 A:
Immunohistochemically staining of HPV-18 E7 protein by goat antiHPV-18 E7 antibodies.



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Figure 21 B :
**Immunofluorescence/ Immunohistochemically staining of HPV-45 E7 protein
by a
combination of antiHPV-16 E7 antibodies and antiHPV-18 E7 antibodies.**



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35/37**Figure 22 :**

**Reactivity of antibodies against HPV18 or 16-E7 proteins in IF.
Weak or unspecific signals O ; strong and specific signals ●.**

	ED17 anti 16E7	N19 anti 18E7	HyT18 anti 18E7	Zym16 anti 16E7	RDI16 Anti 16E7	RDI18 Anti 18E7	Amy16 anti 16E7	Amy18 anti 18E7	Amy16 & Amy18 (combination)
HPV1-E7							●	●	●
HPV6-E7							●	●	●
HPV11-E7							●	●	●
HPV16-E7							●	●	●
HPV18-E7							O	O	O
HPV31-E7									
HPV33-E7							O	O	O
HPV35-E7							O	O	O
HPV39-E7							O	O	O
HPV45-E7							O	O	O
HPV52-E7							O	O	O
HPV56-E7							O	O	O
HPV58-E7							O	O	O
HPV59-E7							O	O	O

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Figure 23A :
Summary of mABs and pAB tested in W-blot and ELISA

Antibody	against	W-Blot: detection of		ELISA: detection of	
		HPV16- E7	HPV18- E7	HPV16- E7	HPV18- E7
BioCarta Camvir 1	HPV16- E1	not spec. -	not spec. -	-	-
BioCarta cocktail	HPV16,18,31E1	not spec. -	not spec. -	-	-
StaCruz ED17	HPV16- E7	+	-	-	-
StaCruz N19	HPV18- E7	-	+/-	minor signal	minor signal
HyTest 18	HPV18- E7	-	not spec. +	-	+
Zymed 16	HPV16- E7	not spec. +	-	minor signal	-
RDI 16	HPV16- E7	+	-	minor signal	minor signal
RDI 18	HPV18- E7	-	not specific +/-	-	minor signal
Amy 16	HPV16- E7	+	-	++	+
Amy 18	HPV18- E7	-	+	+	++
Combin. A 16&18	HPV16E7 HPV18E7	+	+	++	++

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Figure 23B :
Summary of mABs and pAB tested in IF and IHC

Antibody	IF on E7-proteins homologues to		IHC on tumours expressing		
	HPV16 (6 prot.)	HPV18 (5 prot.)	HPV16-E7	HPV18-E7	HPV45-E7
BioCarta Camvir 1	n.d.	n.d.	n.d.	n.d.	n.d.
BioCarta cocktail	n.d.	n.d.	n.d.	n.d.	n.d.
StaCruz ED17	weak (5/6)	-	-	-	-
StaCruz N19	-	weak 4/5 str. 18E7	-	-	-
HyTest 18	-	weak (3/5)	-	½ +	-
Zymed 16	weak (1/6)	-	-	-	-
RDI 16	-	-	-	-	-
RDI 18	-	weak (3/5)	-	½ +	-
Amy 16	strong (6/6)	strong (4/5)	+	-	+
Amy 18	strong (1/6)	strong (4/5)	-	+	+
Combin. A 16&18	strong (6/6)	strong (5/5)	+	+	+